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Development in Tramp Mice

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13. ABSTRACT (Maximum 200 Words) Prospective epidemiological studies indicate that as body weight and/or energy intake increase so does the risk for prostate cancer. In rodent studies chronic calorie restriction is associated with extended life expectancy and decreased incidence of malignancies. A protective effect of energy restriction on development of spontaneous prostate tumors in Lobund-Wistar rats and tumors developing from transplanted prostate tumor tissue cells in mice and rats have been published, but a mechanism of action has not been identified. Recent introduction of the TRAMP (transgenic adenocarcinoma mouse prostate) mouse provides a model that shares characteristics with human prostate cancer. Here, TRAMP mice are being used to evaluate their response to chronic and intermittent calorie restriction. The insulin like growth factor (IGF) axis is being investigated to determine if it is involved in this protective process. Presently, we are following ad libitum-fed, intermittent-restricted and chronic restricted TRAMP mice in a longitudinal study to determine prostate cancer incidence, latency and metastasis rate. A25% reduction in caloric intake is being utilized. Mice also are being enrolled in a cross-sectional protocol. Results will determine if the manner of caloric restriction modulates its protective action and possibly identify a metabolic pathway to target for prevention and/treatment strategies.				
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Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	8
Reportable Outcomes.....	8
Conclusions.....	8
References.....	9
Appendices.....	10

INTRODUCTION:

A number of prospective epidemiological studies indicate that as body weight and/or energy intake increase so does the risk for prostate cancer. In rodent studies chronic calorie restriction is associated with extended life expectancy and decreased incidence of many malignancies. Due to a lack of suitable animal models of prostate cancer, only a few studies have addressed issues of nutrition intervention in the progress of this disease. However, results of these studies support a protective effect of energy restriction on spontaneous prostate tumor development in Lobund-Wistar rats (1;2) and on transplanted tumor/cell prostate tumor growth in mice and rats (3), although a mechanism of action has not been identified. There are limitations to the application of these models to the human disease process. Recent introduction of the TRAMP (transgenic adenocarcinoma mouse prostate) mouse provides a model that shares many characteristics with human prostate cancer (4;5), but their use in nutritional studies has been limited. We are using these TRAMP mice to evaluate their response to chronic calorie restriction, as well as to intermittent caloric restriction/refeeding. These studies are based on our recent report that these two interventions resulted in decreased incidence and extended latency of oncogene-induced mammary tumors in MMTV-TGF- α female mice (6). Furthermore, we found that the intermittent caloric restriction/refeeding regimen was more protective than chronic restriction. TRAMP mice are being followed to determine their response to these interventions with respect to age of prostate cancer detection and metastases rates. Serum and tissue samples will be obtained to determine the role of the insulin-like growth factor (IGF) axis in the protective action of caloric restriction.

BODY:

Progress in relation to Revised Statement of Work 2/13/13 (attached)

TASK 1 & 2. Establish breeding colony & set up genotyping assay.

MONTH 0-3. Order 6 male TRAMP mice (this is the maximum number that can be ordered at one time) and 25 nontransgenic female mice for breeding. Set up breeding. Set up genotyping assay and genotype mice produced. Rebreed mice to expand breeding colony.

Progress on TASK 1 & 2. Breeding stock of TRAMP mice was obtained from Jackson Laboratory and the genotype assay set up as planned. Initially, obtaining offspring was deterred because the mothers were having problems maintaining two litters, *i.e.*, one older ~21 days of age and a new litter born as had been customary breeding procedure in our animal facility (and at Jackson Laboratory). They were abandoning the new litter. Upon consultation with Jackson Laboratory and working with the animal facility personnel we decided to remove the older litter prior to the birth of the new litter. This has worked much better and productivity is presently what we would expect.

TASK 3. Breed mice for EXPERIMENT 1A- LONGITUDINAL STUDY.

MONTHS 4-6. Breed mice to produce one third to one half of mice needed for this study. If one estimates 8 pups per litter, 1 out of 4 pups will be TRAMP males = two TRAMP males per litter. We will need a total of 160 TRAMP males = 80 litters. Genotype offspring. Assign mice to experimental groups. Set up immunohistochemistry assays.

Progress on TASK 3. During this phase we concentrated on enrolling mice in the Longitudinal Study as we were running behind schedule due to the problem cited above. Due to concentration on genotyping and taking care of mice no other assays were set up.

TASK 4. Complete enrollment of mice for EXPERIMENT 1A and 1B SERIAL STUDY.

MONTHS 7-12. Continue breeding to complete EXPERIMENT 1A. Three to four rounds of breeding will probably be needed to supply enough mice. Genotype mice as they are produced. Assign mice to

experimental groups. Once longitudinal study is complete begin assigning mice to serial study for EXPERIMENT 1B.

Progress on TASK 4. Almost all mice are entered in the Longitudinal study (Table 1) and mice are being assigned assigned to a Cross-sectional group which will be euthanized in the summer when we have a summer intern involved in the project (60 mice so far).

Table 1 – Mice Enrolled in Experiment 1A- Longitudinal Study (4/30/04)

	ENROLLED TRAMP MICE (enrolled nonTRAMP mice for age- matched comparisons)	TRAMP MICE TO BE ENROLLED (enrolled nonTRAMP mice for age- matched comparisons)
AD LIBITUM-FED	27 (12)	13 (3)
INTERMITTENT- RESTRICTED/REFED	80 (33)	0 (0)
PAIR-FED	68(14)	12 (6)
TOTALS	175 (59) [216 carried over]*	25 (9) [34 to be enrolled]

*15 TRAMP mice have been euthanized and 3 nonTRAMP mice for age-matched comparisons.

TASK 5. Follow mice in EXPERIMENT 1A and 1B.

MONTHS 6-21. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When age, tumor size and/or animal condition dictates euthanize mice and perform autopsies. Euthanize nontransgenic age-matched mice to correspond to those TRAMP mice with tumors. Euthanize mice that reach terminal ages of 48 or 50 wk of age. Record results and when study complete do statistical analyses of results.

Progress on TASK 5.

Number of mice enrolled. Presently 276 mice are being followed. Due to caging limitations and the initiation of per diem costs by the Hormel Institute after this project was funded we have slowed down the breeding for the time being to deal with the mice we have.

Food Intake. Food intakes for the mice during specific four week cycles of the experiment are shown in Table 2. By prevention of overeating during the refeeding period we are maintaining an overall degree of restriction of ~75% for the intermittent-restricted and pair-fed groups. This should make interpretation of the results more straight forward as we found previously with female mice that some of them overate relative to the ad libitum fed mice during the refeeding stages resulting in overall caloric restriction being lower than originally anticipated (7) (Cleary et al unpublished).

Table 2. Summary of food intake data (grams/day/mouse) for TRAMP mouse study (4/30/04)

	Ad libitum-fed	Intermittent-Restricted	Pair-Fed
Cycle 1	5.56 \pm 0.79*(n=59)	4.2 (n=113)	4.2 (n=64-74)
Cycle 2	5.45 \pm 0.71 (n=30-38)	4.1 (n=111-113)	4.1 (n=21-48)
Cycle 3	5.21 \pm 0.70 (n=29-30)	3.9 (n=73-109)	3.9 (n=20-21)
Cycle 4	4.86 \pm 0.53 (n=26-29)	3.7 (n=65-68)	3.7 (n=5-20)
Cycle 5	4.45 \pm 0.48 (n=25-26)	3.4 (n=25-43)	
Cycle 6	4.31 \pm 0.37 (n=15-22)	3.3 (n=7-11)	
Cycle 7	4.35 \pm 0.35 (n=9-15)		
Cycle 8	4.18 \pm 0.36 (n=9)		
Cycle 9	4.10 \pm 0.30 (n=6-9)		
Cycle 10			
Cycle 11			
Cycle 12			

*mean grams/day/mouse \pm standard deviations for ad libitum fed.

Body weight curves. The body weight curves for the mice as of 4/30/04 are shown in Figure 1. Since the study is ongoing statistics have not been done. In contrast, to our earlier study in female TGF- α mice the intermittent restricted mice are not regaining weight to reach the body weight attained for the ad libitum-fed mice. However, as indicated above in this protocol we are restricting their intake during the refeeding periods so that they do not "overshoot" the intake of the ad libitum fed mice as we found previously for some of the intermittent mice.

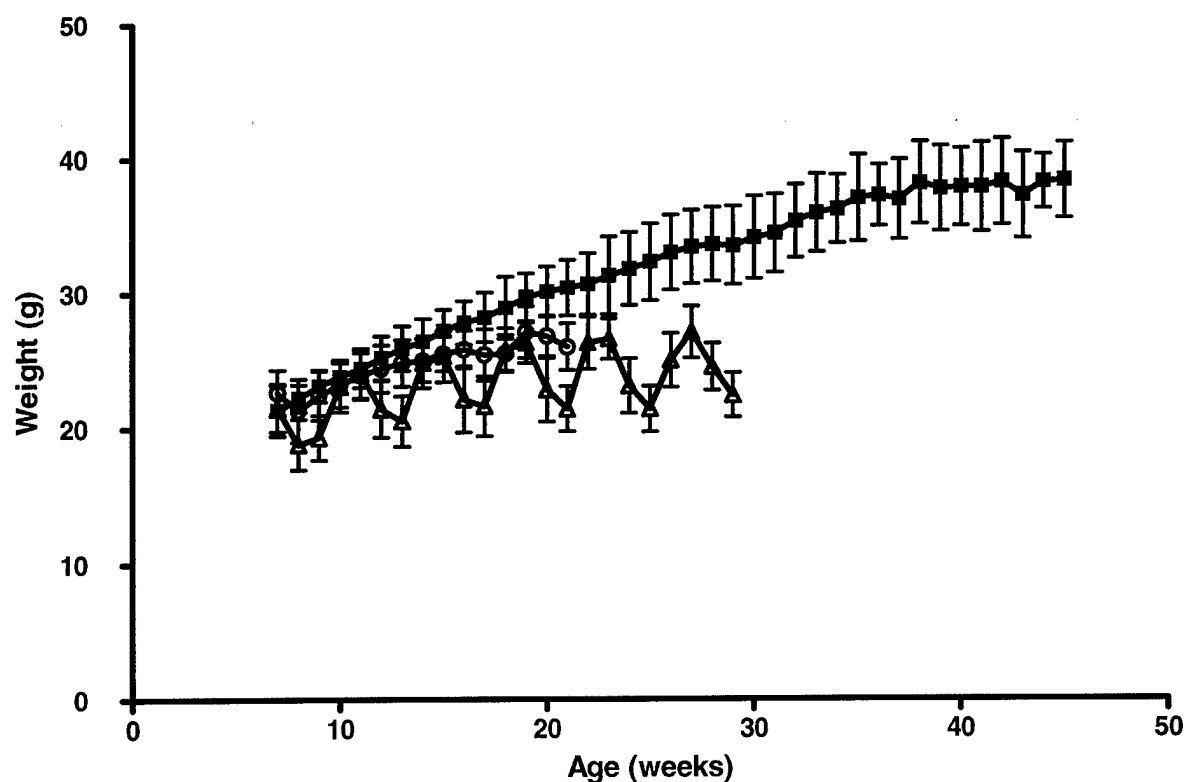


Figure 1: Body Weight curves for male mice in longitudinal study (n is variable at this point due to continued enrollment). No statistics done at this point in time. Black squares = ad libitum; open triangles = intermittent restricted; and open circles = pair-fed.

Numbers euthanized- A list of the mice euthanized to date is shown in Table 3. Nine ad libitum-fed TRAMP mice have been euthanized all due to apparent prostate tumors except for one mouse with hydrocephaly. Two age-matched nonTRAMP mice also have been euthanized. Five intermittent restricted TRAMP mice have been euthanized. Two for apparent prostate tumors and three stopped eating but had no apparent tumors as did one nonTRAMP intermittent restricted. One chronic restricted mouse was euthanized due to tumor on his neck. We are still waiting for most of the pathology reports from these mice.

Table 3. Sacrificed TRAMP and non-TRAMP mice

Mouse #	TRAMP or NON-TRAMP	Feeding	Age when tumor first felt	Age at sacrifice
M5	TRAMP	Ad libitum	34 weeks	44 weeks
M6*	TRAMP	Ad libitum	No prostate tumor, hydrocephaly	18 weeks
M7*	TRAMP	Ad libitum	tumor not detected until dissection, euthanized for lethargy and ↓eating	22 weeks
M8*	Non-Tramp	Ad libitum	No prostate tumor: age-matched control	22 weeks
M12*	TRAMP	Ad libitum	23 weeks	28 weeks
M14*	TRAMP	Ad libitum	22 weeks	26 weeks
M15	TRAMP	Ad libitum	No prostate tumor, euth for non-healing skin infection	29 weeks
M16	TRAMP	Ad libitum	28 weeks	31 weeks
M22	TRAMP	Ad libitum	25 weeks	28 weeks
M23	TRAMP	Ad libitum	18 weeks	23 weeks
M25	Non-TRAMP	Ad libitum	No prostate tumor: age-matched control	28 weeks
M34	TRAMP	Intermittent Restricted	22 weeks	27 weeks
M36	TRAMP	Intermittent Restricted	No prostate tumor, quit eating and drinking	27 weeks
M44	TRAMP	Intermittent Restricted	22 weeks	27 weeks
M80*	Non-TRAMP	Intermittent Restricted	No prostate tumor, euthanized because of lethargy and ↓ eating	9 weeks
M85	TRAMP	Intermittent Restricted	No prostate tumor, quit eating and drinking, ↓ balance—possible inner ear infection	22 weeks
M99	TRAMP	Intermittent Restricted	No prostate tumor, quit eating and drinking, ↓ balance—possible inner ear infection	21 weeks
M120	TRAMP	Chronic Restricted	No prostate tumor, tumor on neck	14 weeks

* HISTOLOGY REPORT HAS BEEN RECEIVED

ORIGINAL-TASK 6. Oncogene and tumor suppressor assays.

MONTHS 6-21. Order supplies and set up assays to perform p53, ErbB2 and possibly other growth factors for determination of gene expression and protein levels Complete setting up assays and analyze samples as they become available. (reviewers indicated not to do this)

REVISED-TASK 6. IGF-BP and IGF-I receptors. Since we are focusing on IGF metabolism any work relating to gene expression will concentrate on factors related to IGF-I action such as IGF-I, IGF-I receptors and IGF-BP's.

Progress on Task 6. The original postdoctoral fellow Xin Hu who was to work on this project left to accompany her husband to Penn. State Medical College prior to the beginning of the study. Despite having received over 100 applications for the position most of the remaining applicants did not have the appropriate background for the position. The few that were qualified were not available by the time I contacted them. I therefore went ahead and hired a well qualified individual at the technical level, Melissa Bonorden to get the study underway. She has been genotyping the mice and monitoring their food intake and health status. A new postdoctoral search was initiated and more successful. Dr. Olga Rogozina who has both an M.D. and Ph.D. will join our research group as soon as she receives permission to work from INS and I anticipate that we will make rapid progress in establishing work on IGF aspects of this work.

KEY RESEARCH ACCOMPLISHMENTS: Establishing the breeding colony, setting up genotyping assay and producing offspring. Enrolling mice in the caloric restriction protocols and following their progress.

REPORTABLE OUTCOMES:

There are none yet. We hope to have enough data to submit abstracts for presentation at the AACR 3rd Annual Frontiers in Cancer Prevention Conference scheduled for October, 2004.

CONCLUSIONS: At this point there are no conclusions that we can present as it is too early to determine outcomes.

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REVISED STATEMENT OF WORK PC020457 2/13/03

Eliminating Specific Aim 5 primarily affects Original Task 6. If there is the opportunity to explore tissue analyses as indicated below it will be focused on aspects of IGF metabolism.

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REVISED-TASK 6. IGF-BP and IGF-I receptors. Since we are focusing on IGF metabolism any work relating to gene expression will concentrate on factors related to IGF-I action such as IGF-I, IGF-I receptors and IGF-BP's.

TASK 7. Restock breeding colony.

MONTHS 12-14. Evaluate breeding colony status and initiate breeding for EXPERIMENT 2-FASTING/REFEEDING study.

TASK 8. Enroll mice in FASTING/REFEEDING STUDY.

MONTHS 14-21. Breed mice, genotype offspring and enroll mice in FASTING/REFEEDING study. For this study 80-120 mice will be needed depended upon adding a PAIR-FED or a RESTRAINED group. We will have to follow the eating pattern of the FASTING/REFEEDING group for several months to determine if the additional group is needed.

TASK 9. Serum and tissue analyses and data analyses.

MONTHS 21-24 Complete tissue assays and when all animals are euthanized perform serum analyses and then complete data analyses of EXPERIMENT 1A and 1B.

TASK 10. Manuscript preparation for EXPERIMENT 1A and B.

MONTHS 25-26 Complete manuscript for the first experiment

TASK 11. Follow mice in FASTING/REFEEDING STUDY.

MONTHS 16-30. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When tumor size dictates kill mice and perform autopsies. Kill nontransgenic mice to be age-matched to mice with tumors. Kill mice upon reaching 50 wk of age if still alive.

TASK 12. Analysis of tissue samples from FASTING/REFEEDING STUDY.

MONTHS 20-32. Perform assays on tumor and normal tissues from FASTING/REFEEDING STUDY as they become available.

TASK 13. Serum and tissue analyses of FASTING/REFEEDING STUDY.

MONTHS 28-32. Determine serum analyses from FASTING/REFEEDING as study groups are completed. Complete tissue analysis.

TASK 14. Complete statistical analysis of data from FASTING/REFEEDING STUDY.

MONTHS 33-34. Complete statistical analysis of data obtained from the FASTING/REFEEDING STUDY.

TASK 15. Prepare manuscript from FASTING/REFEEDING STUDY.

MONTHS 35-36. Write manuscript from results obtained from FASTING/REFEEDING STUDY.